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Studying bats using a One Health lens: bridging the gap between bat virology and disease ecology

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ABSTRACT Accumulating data suggest that some bat species host emerging viruses that are highly pathogenic in humans and agricultural animals. Laboratory-based studies have highlighted important adaptations in bat immune systems that allow them to better tolerate viral infections compared to humans. Simultaneously, ecological studies have discovered critical extrinsic factors, such as nutritional stress, that correlate with virus shedding in wild-caught bats. Despite some progress in independently understanding the role of bats as reservoirs of emerging viruses, there remains a significant gap in the molecular understanding of factors that drive virus spillover from bats. Driven by a collective goal of bridging the gap between the fields of bat virology, immunology, and disease ecology, we hosted a satellite symposium at the 2024 American Society for Virology meeting. Bringing together virologists, immunologists, and disease ecologists, we discussed the intrinsic and extrinsic factors such as virus receptor engagement, adaptive immunity, and virus ecology that influence spillover from bat hosts. This article summarizes the topics discussed during the symposium and emphasizes the need for interdisciplinary collaborations and resource sharing.

KEYWORDS bats, virology, disease ecology, immunity, ASV 2024, satellite symposium

Across the mammalian order, bats and rodents are the most diverse (1), with the bat order (Chiroptera) consisting of more than 1,470 species in over 20 families (2). Bats are keystone species of global ecosystems and perform essential ecological roles such as pollination, seed dispersal, and pest control. However, some bat species are also recognized as reservoir hosts of zoonotic viruses. These viruses include filoviruses [e.g., Ebola virus (EBOV) and Marburg virus (MARV)], henipaviruses (e.g., Hendra virus and Nipah virus), coronaviruses (CoVs) (e.g., SARS-CoV-like, SARS-CoV-2-like, and MERS-CoV-like viruses), and lyssaviruses (e.g., rabies virus) (Fig. 1A). Interestingly, bat species infected with MARV and Nipah virus (3, 4), or other closely related viruses, experience low levels of observable pathology and do not show clinical signs of disease, although some exceptions exist. Tacaribe virus causes a fatal infection in experimentally infected Jamaican fruit bats (*Artibeus jamaicensis*) (5), rabies virus can cause lethal infection in experimentally infected common vampire bats (*Desmodus rotundus*) (6), and Lloviu virus (LLOV) is speculated to cause lethal disease in infected Schreibers' long-fingered bats (*Miniopterus schreibersii*) (7). Thus, understanding the molecular factors that enable bats to better tolerate viral infections (Fig. 1B), along with determining extrinsic ecological factors that influence virus shedding in bats (Fig. 1C), will inform strategies to prevent virus spillover and future consequential outbreaks and pandemics.

Holistically studying factors that influence virus infection and shedding in bats will be best accomplished using a One Health approach. One Health is a concept that recognizes and emphasizes the interconnectedness of animal, human, and environment health. The

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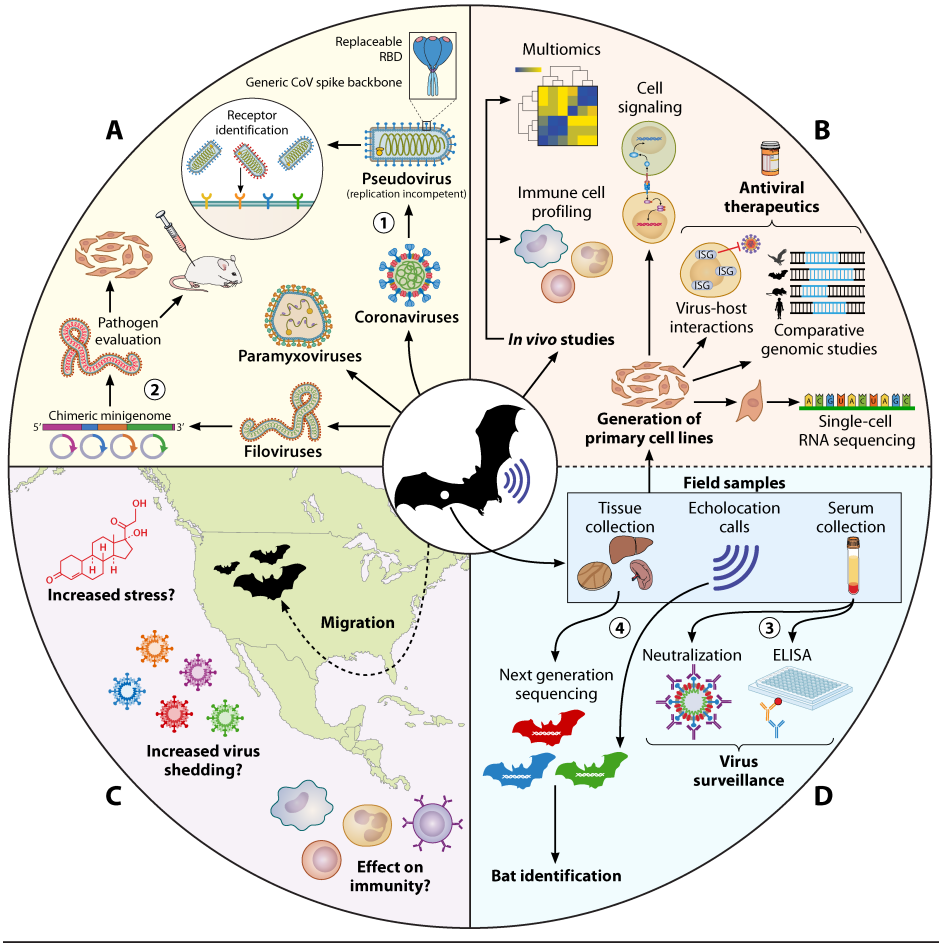

FIG 1 Studying bats using a One Health lens. (A) Some bat species are recognized as reservoir hosts of zoonotic viruses, including filoviruses, paramyxoviruses, and CoVs. The interaction of the CoV spike protein with cellular receptors is primarily mediated through the RBD. (1) Replication incompetent, vesicular stomatitis virus (VSV) pseudotyped viruses engineered with a generic CoV spike backbone and a replaceable RBD have been used to identify cellular receptors utilized by various sarbecoviruses and merbecoviruses. (2) Reverse genetics is an alternative approach to study the pathogenic potential of newly emergent viruses. Mühlberger et al. have developed this approach to generate infectious clones for LLOV, where a chimeric minigenome system complementing the missing genome ends of LLOV with the homologous regions from closely related filoviruses was used. LLOV isolates were used to evaluate virus replication in human and bat cell lines, along with the evaluation of pathogenicity in animal models. (B) Despite harboring viruses that are pathogenic in humans, infected bats do not show overt signs of disease. Research on bat immunity can help us understand the molecular factors that are involved in viral tolerance. One approach to studying bat immunity is the use of comparative genomic techniques and mechanistic characterization of cell signaling pathways and virus–host interactions. These approaches have demonstrated that some bat species have positively selected for genes within non-immune gene containing loci of their genomes to tolerate virus infections. The adaptation of new technologies to study bat immunology, such as scRNA-seq, has been critical for investigating immune cell populations without the need for cross-reactive reagents. (C) An important factor to prevent virus spillover from bats is understanding the intrinsic and extrinsic ecological factors that affect virus shedding in these mammals. Long-distance migration may function as a stressor for bats, as the energetic cost for undertaking such movements could weaken immune function. Migration occurs across bat families, and field studies that interrogate diverse metrics of glucocorticoid and immune activity across the annual cycle are needed to better understand how long-distance movements impact stress physiology, immune activity, and virus shedding in migratory bats. (D) Biosurveillance of bat populations is crucial for identifying and monitoring host species that may harbor pathogens. (3) Sero-surveillance, whether through detection of binding or neutralizing antibodies, can be a powerful tool for emerging zoonotic virus surveillance, circumventing challenges of detecting viral nucleic acid or virus isolates from sub-clinically infected wildlife hosts. Laing et al. have developed antigen-based multiplex serology assays to detect zoonotic viruses in bats, non-human animals, and humans

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Fig 1 (Continued)

and are constructing sero-epidemiology models to elucidate spillover drivers. (4) Accurate identification of host species is essential for effective biosurveillance. DNA barcoding is a common molecular method for species identification, involving voucher specimen which can additionally be used for the generation of cell lines for *in vitro* work. The accuracy of DNA barcoding can be enhanced by ancillary ecological data, such as echolocation calls.

concept of One Health is particularly relevant for zoonotic pathogens, including viruses that originate in bats. For example, a recent study by Eby et al. (8) demonstrated that land-use change and climate change are altering bat residency, which is driving food shortages and clusters of virus spillover.

During the bat satellite symposium held at the 2024 American Society for Virology meeting, bat virologists, immunologists, and disease ecologists came together to share updates on their research, followed by a panel discussion on future directions for the fields. Here, we summarize key discussions from the satellite symposium (Table 1).

BATS AND THEIR VIRUSES

Since the emergence of SARS-CoV in 2002, MERS-CoV in 2012, and SARS-CoV-2 in 2019, thousands of related CoVs have been identified by genome sequencing of samples collected from diverse wildlife species and geographic locations (9–14). Unfortunately, due to the multitude of challenges with isolating viruses, many of the discovered viruses remain uncharacterized, leaving critical knowledge gaps about their potential to infect humans (15). To address this seemingly intractable problem, Letko et al. (15–18) developed a scalable approach to study CoV spike-mediated cell entry, which remains critical to understanding a virus' ability to jump cross-species molecular barriers (Fig. 1A). Since the interaction with cellular receptors is primarily mediated through the receptor-binding domain (RBD) within the CoV spike protein, a generic CoV spike backbone with a replaceable RBD was generated (17). Unlike the whole spike, the gene sequence for an RBD can be synthesized in 4–6 days for as little as US$120. With this approachable and cost-effective tool, Letko et al. (17) tested almost a dozen RBDs for less than the cost of synthesizing one full CoV spike gene. In their proof-of-concept study with the *Sarbecovirus* subgenus, which includes SARS-CoV and SARS-CoV-2, they developed a panel of chimeric spikes with RBDs from 30 sarbecoviruses representative of every natural, unique RBD that has been published to date (17). This panel revealed large numbers of viruses with human cell compatibility—some with known receptors and some with completely unknown routes of entry. The approach was also rapid, allowing for the characterization

TABLE 1 2024 American Society for Virology bat satellite symposium program

Speaker	Session title
Elke Mühlberger	Assessing pathogenic potential and risk factors of newly discovered bat-derived filoviruses
Michael Letko	Functional viromics of betacoronavirus entry
Hannah Frank	Virus-driven selection and immunogenic evolution in bats
Eric Laing	Sero-surveillance as a tool to identify most probable bat hosts and population-level prevalence of zoonotic viruses
Kendra Phelps	Capturing ancillary ecological data during field biosurveillance – getting the most bat for your buck
Daniel Becker	Bat long-distance migration, immunity, and viral dynamics in the wild
Vincent Munster, Stephanie Seifert, Tony Schountz, and Arinjay Banerjee	Open discussion on where the bat virology and disease ecology fields are headed and how we may synergize our scientific efforts

of the SARS-CoV-2 receptor in the laboratory, without having to acquire a virus isolate or patient samples, just 12 days after the genome was published in January 2020 (17).

In their most recent work, Letko et al. (16) applied this concept of testing synthesized spike fragments from the *Merbecovirus* subgenus, which includes MERS-CoV. Merbecoviruses have been discovered in more bat species and over a wider geographic range compared to sarbecoviruses, potentially representing a greater zoonotic threat. The high sequence diversity among merbecoviruses is a significant challenge to the chimeric spike approach because there are few conserved amino acid stretches that flank the RBD in viruses of this subgenus. However, a pair of conserved glycine residues that delineated the exchangeable domain was identified, effectively allowing for the production and testing of 35 chimeric spikes representative of the published diversity for merbecoviruses (16). Screening of this panel against human and animal orthologues of known CoV receptors revealed known and new virus receptor interactions. *Merbecovirus* RBD clades were identified based on spike sequences and entry into Vero E6 and Huh-7.5 cell lines, resulting in four clades that can be applied to describe any merbecovirus. Clade 1 viruses use dipeptidyl peptidase IV (DPP4), clade 2 viruses use human and orthologous angiotensin-converting enzyme 2 (ACE2) in reservoir species, clade 3 viruses use only orthologous ACE2 in reservoir species, and several clade 4 RBDs use an unknown receptor to infect human cells (16). ACE2 was identified as the receptor for the entire HKU5 complex of merbecoviruses, which has been an elusive virus–host interaction in the field for almost 20 years (16). Importantly, this information provides a new framework for studying merbecoviruses and the evolution of their diverse receptor usage. This ongoing work will also identify merbecoviruses that carry the highest level of human cell compatibility, which is crucial for pandemic preparedness and broad-spectrum therapeutic design.

In addition to CoVs, some bat species are reservoir hosts of filoviruses, a group of negative-sense RNA viruses. Some members of the *Filoviridae* family, including EBOV, Sudan virus, and MARV, cause severe disease in humans with high case fatality rates (19). Due to recent advances in high-throughput sequencing technologies, genomic sequences of unknown filoviruses have been found in various vertebrate species, including bats, snakes, and fish (7, 20–25). This includes LLOV that was first detected in carcasses of Schreibers' bats in Spain in 2002 (7, 26), with recent isolation of the virus from Schreibers' bats in Hungary in 2022 (27, 28). The emergence of LLOV in Spain and Hungary correlated with unexplained increased mortality among Schreibers' bat colonies, including signs of respiratory distress, but it remains unclear if the fatalities were caused by LLOV infections (7, 29). The close relationship of LLOV to the highly pathogenic EBOV and MARV raises questions about its pathogenic potential for humans.

There are several challenges to studying the pathogenic potential of newly emergent viruses, such as LLOV. First, there might only be sequence information available but no isolates for infection studies. Second, while reverse genetics tools can be used to generate infectious clones based on published sequences, these sequences can be incomplete or erroneous. To address these issues, Mühlberger et al. (30) established a chimeric minigenome system for LLOV, where the missing genome ends for LLOV were complemented with the homologous regions from closely related filoviruses (Fig. 1A). Based on the minigenome results, recombinant infectious LLOV (rLLOV) clones were generated and used to evaluate pathogenicity (Fig. 1A). Studies with rLLOV also included assessment of host and cell tropism, replication efficiency, and innate immune signatures in critical target cells of filovirus infection, including Schreibers' long-fingered bat-derived kidney cells (SuBK12-08) and human-derived cell lines (31). Mühlberger et al. demonstrated that rLLOV can infect human cells, but it replicates slowly and fails to induce an inflammatory response in macrophages (31), in which the induction of an uncontrolled inflammatory response is a hallmark of fatal EBOV disease. These data were further supported by infection of interferon-α/β receptor knockout mice with authentic LLOV isolated from Schreibers' long-fingered bats in Hungary (27), which did not show signs of disease (32). Together, these data suggest that LLOV poses a low

risk to human health. Furthermore, these data suggest a tractable workflow to assess the pathogenic potential of newly emergent viruses and identify bottlenecks, such as missing or erroneous sequence information that hamper work on these viruses.

BAT IMMUNITY

Compared to humans and mice, understanding of bat immunology is limited in part because of species diversity and a lack of animal models, resources, and reagents (Table 2) (33). Much of the focus on bat immunology has been centered around the innate immune system (34–43). Additionally, a number of comparative genomic studies and functional characterizations have shown that bats have positively selected for genes within the non-immune loci of their genomes that are thought to help resist viruses (44–47). Although findings vary greatly between pathways and bat species, taken together, these studies suggest that bat immunity shares many of the fundamental signaling pathways that are found in humans, mice, and other mammals, with bat-specific adaptations that may aid in their resistance to or tolerance of viruses (48). However, even with these studies, much remains to be learnt about virus–host interactions in bats. Knowledge about the adaptive immune system of bats, especially B- and T-cell subsets and receptor repertoires is particularly limited when compared to knowledge of the bat innate immune system (48–50). Perhaps the biggest challenge in studying bat immunology is understanding the differences in immunity and pathogen response between the over 1,470 bat species. Bats vary widely in their ecology, geography, and viral flora (15, 51–53); all these factors have likely impacted the evolution of antiviral immunity. For example, straw-colored fruit bats (*Eidolon helvum*), found in the same geographic areas as EBOV, may have evolved to be refractory to EBOV infection (44). In addition, ACE2, DPP4, and other host proteins bound by CoVs are widely variable across bats (45). Due to constraints such as a paucity of captive colonies, traditional immunological reagents, and conservation concerns, most knowledge on bat immunity draws from a limited number of species (54).

Trends are changing as researchers adapt new technologies and existing tools to these non-model organisms (Fig. 1B). Next-generation sequencing techniques are often species-agnostic and have proven particularly useful for gaining insights into resistance of bats to infection. Transcriptomics has helped clarify bat responses to infections (61–64) and can be done from field samples (65). Long-read sequencing techniques have enabled the generation of reference bat genomes, along with insights into how gene loss and expansion may have impacted bat immunity and inflammation (66–69). Single-cell RNA sequencing (scRNA-seq) has been particularly useful for investigating immune cell populations without the need for antibodies specific to different cell types that are required in traditional flow cytometry analyses (61, 70, 71). Advances in the qualities of genomes and scRNA-seq have also yielded significant insights into bat B- and T-cell receptor repertoires (49), including the finding that bats in the Vespertilionidae family, the largest bat family, have two, independent, functional immunoglobulin heavy chain loci (72). Similarly, proteomics has provided additional insights into the bat immune phenotype in the wild and in response to viral infection (73–75). Additionally, as interest in bat immunology has increased, more institutions are establishing captive colonies for *in vivo* work, and researchers are creating primary cell cultures from a wider variety of species to test hypotheses *in vitro* (76, 77). These emerging methods will enhance knowledge not only of the fundamental components of bat immune systems, particularly the adaptive immune systems that are not well studied, but also of how conserved these mechanisms are across diverse bat species. Finally, a better understanding of how reservoir bat species better tolerate viral infections may one day pave the path for bat-inspired antiviral therapeutics for humans (54).

ECOLOGICAL PHYSIOLOGY

One of the central hypotheses to explain when and where bats actively shed viruses focuses on the role that physiological stress may play in disrupting tolerance of infection

TABLE 2 Resources available for bat-related research

Resource	Description
Bat1K Project	An initiative to generate and annotate genomes for all living bat species
Bat Conservation International	An organization committed to conserve the world's bats and their ecosystems through research
Bat Eco-Interactions	A platform for scientists to investigate bats and their role in our environment
Bat One Health	A forum for scientists interested in the connections between environmental change and health, where the goal is to understand pathogen emergence from bats
Batnames.org	A dynamic resource for the most up-to-date bat taxonomy, including number of bat species recognized
ChiroVox	The largest public library of bat calls currently available (55)
A Coalesced Mammal Database of Intrinsic and Extrinsic Traits	A comparative data set of ecological and life history traits among mammals (56)
Database of Bat-Associated Viruses	A comprehensive, up-to-date database of viruses detected in bats (57)
DarkCideS 1.0	A global database of bat caves and species, providing geographical location, ecological status, species traits, and parasites and hyperparasites (58)
EuroBaTrait 1.0	A species-level trait database of bats in Europe, including genetic composition, physiology, morphology, acoustic signature, roost type, diet, etc (59)
Global Union of Bat Diversity Networks	A community of bat researchers focused on enhancing research addressing bat diversification and sustainability
Global South Bats	A community of bat researchers in the Global South coming together to find solutions to common bat conservation challenges
The Global Virome in One Network data set	The largest open-access database on vertebrate–virus associations (60)
Latin American and Caribbean Bat Conservation Network	A network of 25 countries in Latin America and the Caribbean that promotes research and bat conservation
North American Society for Bat Research	A group that facilitates communication and collaboration among scientists, educators, and the public to promote the study and conservation of bats
WildTrax	An open data platform for environmental sensors, helping contribute data to the broader NABat program and international assessments
Eptesicus fuscus kidney cells (EfK3B)	Immortalized kidney cell line available through Kerafast (#CVCL_GZ34)
R. aegyptiacus fetal cells (R06E)	Immortalized fetal cell line available through BEI Resources (#NR-49168)
Tadarida brasiliensis lung cells (TbLu–1)	Primary lung-derived cell line available through ATCC (#CCL-88)

(78–80). Early work demonstrated links between stressors such as pregnancy and food scarcity with Hendra virus seropositivity in little red flying foxes (*Pteropus scapulatus*) (81), with more recent work showing food shortages interact with displacement of pteropid bats into novel habitats to predict seasonal pulses of Hendra virus shedding (82). Such patterns are likely explained by linkages among energetic demands, glucocorticoids, and immunity (Fig. 1C). Like any vertebrate, bats use glucocorticoids to increase energy mobilization and reallocation to meet immediate demands, but this can occur at the expense of physiological processes like the immune response (83, 84). However, the immune mechanisms linking stressors and viral shedding in bats remain poorly understood (85), and such work has focused predominantly on food scarcity with less attention to other energetic demands of bats in the wild (86, 87).

Long-distance migration—defined as the seasonal, two-way movement of individuals between reproductive and wintering grounds (88)—has been established as a stressor in other taxa such as birds (89), where the energetic costs of preparing for or undertaking such movements can weaken immune function and allow chronic infections to reactivate (90, 91) (Fig. 1C). In contrast, such impacts of migration for bats have not been adequately explored. Migration occurs across bat families but is most common in the Vespertilionidae and Molossidae, where certain species seasonally migrate between 100 and 2,000 kilometers (92, 93). To date, studies of bats have shown that the energy

demands of migration can equal that of reproduction (94), where switching from the pre-migratory to migratory season occurs alongside adjusted allocations in different arms of the immune response (95), and that some measures of immunity differ between migratory and non-migratory individuals (96, 97). Field studies that interrogate diverse metrics of glucocorticoid and immune activity at fine time intervals across the annual cycle are needed to better understand if these long-distance movements function as a stressor in bats. Such work could be further advanced by adopting multi-omics approaches relevant to bats (65, 73) and by ultimately assessing biomarkers of enrichment in relation to viral positivity (74). These studies could then guide experimental approaches, such as by using glucocorticoid levels seen during migration in the field to inform *in vitro* or *in vivo* challenge studies that test effects on immunity and virus replication (33, 85). Methods used to study flying animal movement, including wind tunnels and the Motus Wildlife Tracking System (98), could also be used to quantify how migratory timing and duration affect bat stress physiology, immune activity, and viral shedding.

Animal migration has long been recognized as an important factor in shaping infectious diseases (99), and long-distance migration of bats in particular has the potential to affect where and when human exposure to zoonotic viruses is likely (100, 101). There remains an important need to understand how these seasonal, energetically costly movements affect immunology and the downstream implications for viral transmission and spatial spread (102). Robustly assessing when and how migration influences how bats tolerate zoonotic viruses is critical to better understand virus–host interactions and improve the ability to predict transmission risks.

BIOSURVEILLANCE AND BAT SPECIES IDENTIFICATION

In recent years, Nipah virus, EBOV, and MARV have been detected outside their historic ranges (103–107). While zoonotic transmission events leading to disease outbreaks are rare, these events highlight the challenges of rapid diagnostic confirmation and outbreak mitigation. In practice, there is a limited window of opportunity during which viruses are actively shed from a host. Evidence indicates that certain species of bats, such as *Pteropus* spp. and *Eidolon* spp. are hosts of henipaviruses, and *Rousettus* spp. and *Mops* spp. are hosts of filoviruses (4, 25, 108–117). However, many virus–host relationships remain less-defined, such as the case of EBOV (118, 119), which weakens early warning detection and global health security. Detection of viremia or virus shedding from bats is rare, especially for filoviruses, which are seldom identified via molecular analysis of non-lethally collected blood and mucosal samples. This makes it difficult to detect active infections without knowing *a priori* when they occur. In contrast, wildlife hosts may have detectable antibodies against specific pathogens for months or years following productive infection. Thus, measurement of virus-specific immunoglobulin G (IgG) antibodies provides indirect detection of previous infections and thus an alternate form of zoonotic virus surveillance (Fig. 1D), which can be leveraged to infer viral transmission dynamics in wildlife populations (120). Though it is important to note that in experimentally MARV-infected Egyptian fruit bats (*Rousettus aegyptiacus*), virus-specific IgG antibodies detected by enzyme-linked immunosorbent assay or microneutralization rapidly waned with bats losing detectable neutralizing antibodies in roughly 3 months, thus highlighting potential differences between field- and lab-based studies and perhaps also between bat species and virus types (121).

Most statistical approaches to interpreting serosurveillance data are limited to single-antigen analysis, which limits understanding of bat immunology and virus circulation within bat populations and communities. Furthermore, expected and unexpected antibody cross-reactivity frequently confounds conclusions from both single and multiple-antigen assays. For example, orthoebolavirus-positive antisera are highly cross-reactive with protein antigens from heterotypic EBOVs (122). Antigenic cartography permits visualization and an understanding of the relationship among viruses based on antigenicity instead of phylogenetics (123). Antigenic maps have been widely used

within influenza and SARS-CoV-2 studies to aid in guiding effectiveness of ancestral infection and vaccine-induced immunity against novel variants and strains (124–127). The development of antigenic maps for other priority zoonotic viruses, such as filoviruses and henipaviruses, could aid in establishing expectations of cross-reactivities between genetically characterized viruses (128) and assist in the rapid identification of antigenically novel viruses that are currently genetically undiscovered. Access to and serological testing of confirmed post-infection or post-immunization sera would be a first step toward understanding the serogroup relationships.

Laing et al. (129–131) have developed antigen-based multiplex serology assays to detect and identify zoonotic viruses and develop models that highlight underlying seasonal patterns of virus circulation and drivers of virus release in bat host populations, which are being used in active biosurveillance projects. New approaches such as phage immunoprecipitation sequencing (PhIP-seq) can facilitate a complete antibody profile of an individual's virome (132–134). However, cross-assay comparisons of peptide-based PhIP-seq and antigen-based multiplex tests will be necessary to validate epitope selection and interpretation of sero-surveillance. Ultimately, serosurveillance is useful for building sero-epidemiological models to elucidate the drivers and processes of virus transmission, estimating force-of-infection for zoonotic viruses, and providing valuable prevalence data to develop spatiotemporal spillover distributions and interfaces with sufficient resolution to inform mitigation strategies.

Biosurveillance of bat populations is crucial for identifying and monitoring host species that may harbor pathogens (135). Despite its importance, several gaps hinder the effectiveness of current biosurveillance efforts, particularly validation of the taxonomic assignment of bat species from which diagnostic samples were collected for pathogen screening, limited integration of ancillary ecological data to improve species identification and to provide context for interpreting bat-pathogen dynamics, and a lack of data sharing on publicly accessible databases.

Accurate identification of host species is essential for effective biosurveillance. DNA barcoding is the most commonly used molecular method for confirmation of species identification (136); however, conclusions about species identification based solely on a few hundred base pairs may lack the reliability needed to accurately differentiate closely related species (137). An example of this are two sibling bat species, the lesser mouse-eared bat (*Myotis blythii*) and the greater mouse-eared bat (*Myotis myotis)* (138), which are thought to undergo cryptic hybridization in areas where they coexist, such as Türkiye (139). To enhance the accuracy of species identification in bat biosurveillance, it is essential to complement host DNA barcoding with ancillary ecological data that can be easily collected in a field setting, such as echolocation calls (Fig. 1D). Echolocation calls are species-specific for most bat species and can provide an additional layer of confirmation for species identification (51), which can be particularly useful in areas where barcoding may fall short. Echolocation data can provide valuable insights into bat behavior, such as foraging patterns, roosting habits, and migration routes (51), which is informative for identifying high-risk areas for pathogen transmission. Furthermore, echolocation detectors are more affordable and compact, with some like the Echo Meter Touch (Wildlife Acoustics) costing as little as US$179 and function using most smartphones and tablets, making detectors easily obtainable for bat biosurveillance studies.

A voucher specimen is a preserved whole-body organism and/or associated samples that serve as a verifiable and permanent record of a species occurrence from a specific location and at a specific time (140). The goal is for each voucher specimen to be a holistic specimen, where frozen tissues, as well as biological and diagnostic samples are collected in addition to dried skin and skeleton to maximize the amount of information collected from a single euthanized individual (141). Holistic voucher specimens preserved during field sampling and deposited for long-term storage in curated collections represent a pre-existing tool that can aid in pandemic preparedness, in addition to the identification of host species when zoonotic spillover events occur

(Fig. 1D) (142). If voucher specimens are preserved during field sampling, the origin of zoonotic diseases and viral spillover events could be more accurately and easily assessed. For infectious disease research, this ability to verify the host species identity coupled with ancillary ecological data is incredibly important in determining how pathogens can infect humans and the possible routes of transmission (143).

While the collection of diagnostic samples for molecular screening of viruses is often the primary focus of wildlife biosurveillance, taking a One Health approach by collecting ancillary ecological data can better inform virus-host dynamics. Combining host barcoding with echolocation and other ecological data types, such as holistic voucher specimens, with molecular screening for potential pathogens will create a more robust and comprehensive One Health approach to better inform transmission risk and develop intervention strategies (Fig. 1D). Moreover, sharing ecological ancillary data such as depositing voucher specimens in curated collections, publishing echolocation recordings, or uploading species occurrence records (e.g., ~4,300 individual occurrence records from the Western Asia Bat Research project published on the Global Biodiversity Information Facility) in publicly accessible databases benefits the scientific community including wildlife biosurveillance studies (144, 145).

FUTURE DIRECTIONS FOR THE FIELD

The fields of virology, immunology, and disease ecology have made steady progress to better understand disease tolerance and virus transmission in bats. However, during the symposium, it was evident that the lack of interdisciplinary collaborations between the three fields has left critical knowledge gaps that can only be addressed by larger multi-disciplinary studies. For example, studies have identified stressors in wild-caught bats that correlate with virus shedding, but the lack of molecular studies impedes our understanding of how and when infected bats shed viruses. Furthermore, biosurveillance studies have identified multiple novel viruses in bat species, but the lack of mechanistic studies has left gaps in our understanding of viral pathogenesis, transmission, and disease tolerance in these bats and other susceptible species. Indeed, for a holistic understanding of disease tolerance and viral shedding in bats, there is a need to develop large interdisciplinary, collaborative studies, for which we need to develop resource-sharing platforms and funding mechanisms (Table 2).

There is also a need to study viruses and other microorganisms that are pathogenic in bats. Current research efforts are predominantly focused on bat-associated viruses with zoonotic potential. Thus, research on viruses that cause disease in bats, such as Tacaribe virus, is largely limited. In addition, non-viral microorganisms, such as bacteria, have not been extensively studied in bats, although microbiome profiling has been performed in some bat species, and some bacterial taxa (such as bartonellae and mycoplasmas) have been more robustly studied in bats (146–148). Furthermore, there is limited research on the ecological overlap of bat species and other animals. Studying the environment along with the animals and their microbes using a One Health lens will lead to a more holistic understanding of interactions that occur between various animal species, including bats and their microbes, and lead to the discovery of extrinsic factors that influence pathogen shedding in wildlife.

Bat research has been slow over the last two decades largely due to the lack of molecular tools and cross-reactive reagents that would enable researchers to comprehensively study immunity and infection in the over 1,470 bat species. Indeed, the need to develop a resource-sharing platform along with interdisciplinary funding opportunities was strongly highlighted during the closing panel discussion (Table 2). The concluding session once again highlighted the need to bridge the gap between field- and laboratory-based studies to better understand how bats tolerate virus infections, along with discovering and characterizing the intrinsic and extrinsic factors that lead to virus shedding in bats. In summary, the field of bat research is rapidly growing, and this area of research presents a unique opportunity for trainees to hone their skills in applying One Health solutions to zoonosis and wildlife conservation.

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